

Effect of aqueous and alcoholic of *paulownia tomentosa* l. Leaves extract on *casuarina equisetifolia* root rot

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This study, conducted at the Department of Forestry Sciences in the College of Agriculture and Forestry, University of Mosul, aimed to analyze the seasonal distribution of Casuarina root rot disease. Field surveys across private and public nurseries in Mosul during November 2020, January, March, May, July, and September 2021 facilitated the investigation. The highest disease incidence was recorded in May 2021 at 24%, contrasting with a low of 10% in January 2021. The identification of causal agents revealed the presence of *Fusarium solani* and *Rhizoctonia solani*. Notably, *Fusarium solani* exhibited a peak isolation rate of 52% in July 2021, while *Rhizoctonia solani* displayed the lowest rate at 10.33% in January 2021. Furthermore, the assessment of *Paulownia tomentosa* leaf extract's impact on fungal growth inhibition illustrated the superior performance of the alcoholic extract. The alcoholic extract achieved 100% inhibition of *Rhizoctonia solani* growth at the fourth concentration, whereas the aqueous extract exhibited a minimal inhibition rate of 2.50% at the first concentration.

Keywords: Paulownia, leaf Extracts, root rot, fungus and Casuarina, fusarium solani, paulownia tomentosa and disease incidence.

INTRODUCTION

Casuarina is a rapidly growing evergreen tree characterized by scale-like leaves and drooping branches. It reaches a height of 10 to 25 meters, bearing small cones and a straight stem in young trees. The crown starts as conical and transitions to oval with the tree's age. The branches are cylindrical and hang downwards. The small scale-like leaves on the branches play a pivotal role in photosynthesis and significantly influence the tree's essential functions (Diemer, 2004). This tree species is indigenous to the coastal areas of the southeastern coast of India (Mascarenhas and Jayakumar, 2008; Zoysa, 2008).

Casuarina equisetifolia L. is widely distributed in Iraq and is known for its robust growth in flat areas and well-irrigated soils. Its northern expansion is constrained by the rain line extending from the north of Mosul and Erbil. This climatic limitation renders it susceptible to cold and frost, preventing its growth in mountainous regions (Daoudi, 1979). The tree can reach a height of 20 meters in less than 12 years (Balasubramanian, 2005). It finds application primarily in ornamentation and soil preservation, but it's unsuitable for making wooden bars (Hasan et al., 2020). Seedlings of this tree, classified as forest trees, face fungal threats causing root rot, such as *Fusarium sp.*, *R. solani*, and *M. Phaseolina*,

leading to significant economic losses. Other contributors to root rot encompass fungi like *Cylindrocarpon tenue*, *F. Oxysporum*, *Microdochium bolleyi*, *Mucor sp.*, *Pestilopsis funera*, *Phoma pomorum*, and *Pythium sp.* (Muhammad, 1987; Sunderrao et al., 2017; Al-Khairu & Hamid, 2020). With the escalation of plant diseases and concerns about chemical pesticides, several studies endorse the effective use of plant extracts as ecologically safe and highly efficient alternatives for controlling these pathogens (Al-Kaif, 2015). *Paulownia tomentosa*, originally from West Asia and belonging to the family Scrophulariaceae, is predominantly found in China (Kaymakci, 2010). It's a fast-growing deciduous forest tree characterized by a straight vertical stem. It holds economic significance due to the quality of its wood for various industries. Varieties include *P. catalpifolia*, *P. elongate*, *P. fortuneie*, and *p.henan*. Its planting regions extend to South Asia, Australia, Japan, Germany, and Southern Europe (Kaplan, 2008). Paulownia leaves contain secondary compounds, including phenols. Research suggests that the leaves and flowers of *Paulownia tomentosa* contain phenols, playing a pivotal role in combating fungi causing root rot (Özge & Yeşim, 2019). Consequently, this study aims to employ *Paulownia tomentosa* leaf extract as a remedy

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against casuarina seedling root rot, specifically *Casuarina equisetifolia*.

MATERIALS AND METHODS

Field survey of root rot pathogens: A field survey was conducted on private greenhouses in the city of Mosul, along with the greenhouse of the Department of Forestry Sciences, targeting the root rot disease affecting *Casuarina* seedlings aged 1-3 years. This study spanned the months of November 2020, January, March, May, July, and September 2021, aiming to analyze the seasonal distribution of fungi responsible for causing root rot disease in these bag-grown seedlings.

The quantification of *Casuarina* root rot infection involved estimating the percentage of infected seedlings per 100 seedlings. This calculation was based on observed symptoms such as yellowing and vegetative system decay. To ensure accurate infection determination, samples were randomly collected, and subsequent examination of the root system was performed. Notably, symptoms were observed on both the external roots and root hairs.

The infection percentage was determined as follows:

$$\text{Infection \%} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Isolation: Samples of infected *Casuarina* seedlings were collected during the survey period from the greenhouses. Randomly selected samples were taken from seedlings grown in agricultural nylon bags (polyethylene) and subsequently rinsed under running water for a duration of 4 hours. This rinsing was performed to eliminate dust adhered to the roots as well as any foreign debris. Following the rinsing process, the roots were sectioned into segments measuring 1-0.5 cm in length. These segments were then subjected to superficial sterilization, involving immersion in a 1% solution of sodium hypochlorite (NaOCl) for a period ranging from 3 to 4 minutes. Subsequently, the segments underwent a thorough washing with sterile distilled water. After the washing, the segments were carefully dried using two sterile filter papers. The dried segments were then placed onto sterile Petri dishes with a diameter of 9 cm, each containing a nutrient medium known as Potato Dextrose Agar (PDA) infused with the antibiotic streptomycin sulfate. The concentration of streptomycin sulfate was maintained at 50 mg/l to inhibit bacterial growth before the medium solidified. Four segments were placed in each Petri dish. The dishes were subsequently incubated at a temperature of 25±2 degrees Celsius. The results were acquired by quantifying the number of fungal colonies present in each dish. This numerical data was then converted into a percentage representing the isolated fungal colonies. The isolated colonies, obtained through the Hyphal tip method (Riker & Riker, 1936), were purified and characterized. Microscope slides were prepared, and the fungi were examined at two magnification levels: x10 and x40. The

diagnosis of isolated fungi was based on species identification utilizing the characteristics of the colony, fungal hyphae, spores, and their compositions. This taxonomic classification was guided by globally recognized taxonomic keys established by (Parmeter & Whitney, 1970; Barnett & Hunder, 1972; Booth, 1977). The selected fungal strains were preserved within test tubes containing an inclined agar (slant) medium at a temperature of 5 degrees Celsius. These preserved samples were reserved for subsequent tests. The isolation process was conducted at two-month intervals for a year, considering the plant species under study.

The percentage of isolated fungi was calculated using the following methodology:

$$\text{Isolated Fungus \%} = 100 \times \frac{\text{Number of isolated fungus colonies}}{\text{Total number of developed colonies}}$$

Pathogenetic Ability Test for Isolated Fungi: The pathogenicity of these fungi was assessed following their isolation from infected *Casuarina* seedlings, employing the method outlined in (Leslie & Summerell, 2006). Healthy seedlings, aged 1-3 years, were cultivated in agricultural bags with a capacity of 3 kg. *Casuarina equisetifolia* was grown in the greenhouse of the Department of Forestry Sciences, College of Agriculture and Forestry, University of Mosul. To initiate the experiment, incisions were made on the roots of these healthy seedlings using a sharp, sterilized scalpel. Afterwards, the fungi designated for the test were prepared. A growth medium was formulated utilizing potato agar and dextrose (PDA), which was then placed in Petri dishes with a diameter of 9 cm. Furthermore, the nutrient medium was inoculated with fungal mycelium using an electric mixer of the Go Sonic type. Each healthy seedling was provided with a Petri dish, and the medium was mixed with the root soil. This experimental setup comprised 4 replicates, with each replicate containing 4 seedlings. The assessment was conducted 75 days after the pollination process. Subsequently, the seedlings were carefully uprooted and subjected to a thorough washing using running water. The infection percentage was determined for each individual fungus affecting *Casuarina* seedlings using the following formula:

$$\text{Infection \%} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

To assess the impact of pathogenic fungi on various growth attributes of *Casuarina* seedlings, measurements were taken for the overall vegetative height, root system length, wet weight, and subsequently, dry weight following a 48-hour drying period in a 70°C oven (Taha *et al.*, 1986). The experimental design employed was a Randomized Complete Block Design (RCBD), with data analysis performed using the Dunkin' polynomial method.

Preparation of raw materials and collection of sample: Samples were collected from mature leaves of insect- and disease-free *Paulownia tomentosa* trees originating from various locations across the University of Mosul campus,



following the methodology outlined in (Browning, 1967). These leaves were carefully placed into nylon bags, then transported to the laboratory for thorough washing to eliminate dust particles. Subsequently, the plant leaves were air-dried in the shade, cut into smaller fragments, and left to naturally dry for a span of 21 days until a consistent weight was achieved. Using an electric grinder (home-built), the dried leaf fragments were ground into smaller particles. These particles were then sifted through a 60-mesh sieve after passing through a 16-mesh sieve. This refined sample was primed for extraction (Harborne, 1973)

A - Production of Alcoholic Leaf Extract: The extraction method as described by (Browning, 1967) was adopted, with ethanol (95%) serving as the solvent. In adherence to the technique specified in (Harborne, 1973), 50 g of ground leaf powder was placed in a 500 ml glass flask. Subsequently, 500 ml of 95% ethanol was introduced. A magnetic stirrer facilitated the agitation of the mixture for a 48-hour period. The solution was then filtered using layers of medical gauze, and further filtration through Whatman No.1 filter paper followed suit. The remaining content was subsequently concentrated using hot water extraction. The leaf extract was subjected to concentration in a rotary vacuum evaporator at a temperature ranging between 40-50 °C, yielding 25 ml of unprocessed extract. This filtrate was then stored in opaque containers within a refrigerator at 4°C until further biological tests were conducted (Khanzada *et al.*, 2006).

B - Production of Hot Aqueous Leaf Extract: A consecutive extraction technique was employed to separate sample components based on their polarity. The previously ethanol-extracted residual sample was reused. The material was dried through air exposure and then placed within a one-liter glass container. Approximately 400 ml of hot water at 80°C was introduced into the container. Continuous stirring utilizing an electric stirrer was performed over a 24-hour duration, with intermittent cooling of the solution. Filtration was executed using muslin cloth (medical gauze) to eliminate larger impurities. The filtrate was subsequently subjected to filtration through Whatman No.1 filter paper, followed by air drying under laboratory conditions. This process yielded aqueous extract powder, which was preserved in sealed opaque bottles for future biological testing.

BIOTESTS

The Impact of Alcoholic and Aqueous Extracts on Fungal Growth Inhibition: Sterile PDA nutrient medium was employed to prepare 100 ml containers. These containers were subsequently infused with varying concentrations of alcoholic and aqueous extracts. Prior to solidification, plant extract powder (1 g) was meticulously dissolved in 10 ml of Dimethyl Sulfoxide (DMSO). Similarly, the aqueous extract was also prepared, with concentrations of 25%, 50%, 75%, and 100%. This was coupled with a comparison treatment absent of extracts. Subsequently, the mixture was introduced into sterile Petri dishes with a diameter of 5 cm. Following

solidification, fungal isolates, obtained from the tip of colonies via the HAEVA (Hyphal tip method) (Riker & Riker, 1936), were placed at the center of each dish using tablets with a 5 mm diameter.

Each treatment was comprised of 4 replicates, with each replicate encompassing 3 Petri dishes. Additionally, the comparison treatment was also included in the experiment. The dishes were then subjected to incubation at a consistent temperature of 25±2°C. The results were recorded once the fungal mycelium had filled the comparison dishes, involving the calculation of the average measurement derived from two perpendicular colony diameters. Subsequently, the growth inhibition ratio was calculated utilizing the following formula:

Growth inhibition% = Average colony diameter measurement for comparison – average colony diameter measurement of the transaction / Average measure of colony diameter for comparison × 100

A factorial experiment was conducted using a Complete Random Design (CRD), involving three factors. The first factor encompassed various fungi, the second factor focused on the type of extract used, and the third factor pertained to the concentrations of the extracts. Statistical analysis was applied to the results, and the Dunkin' polynomial method (Alraawy *et al.*, 2000) was employed for testing purposes.

RESULTS AND DISCUSSION

Field survey of cypress seedling root rot disease: The outcomes of the field survey exhibited fluctuations in the incidence of root rot disease among casuarina seedlings, illustrated in Figure 1. The highest incidence rate, observed in May 2021, reached 24%, followed by a subsequent 21% in July of the same year. The drop in disease percentage during July can be attributed to temperatures exceeding the fungi's viable range, coupled with shifts in humidity levels. Additionally, the incidence rate exhibited a relatively lower value of 16% during November 2020, whereas the lowest incidence of root rot disease was recorded at 10% in January 2021. The reduced infection rate in January can be attributed to the colder temperatures prevailing during that month.

The infection rates align logically with the management practices in these nurseries. It's important to acknowledge that these nurseries necessitate preventive and curative protocols to curb the proliferation of root pathogens (Taha *et al.*, 1986). Recommendations for the essential control and maintenance of these nurseries are imperative. These results corroborate findings reported by (Muhammad, 1987) extending beyond the borders of Iraq. The global prevalence of this disease is evident, exemplified by high infection rates among forest trees, as observed in Indian Kashmir (Ahanger *et al.*, 2011)



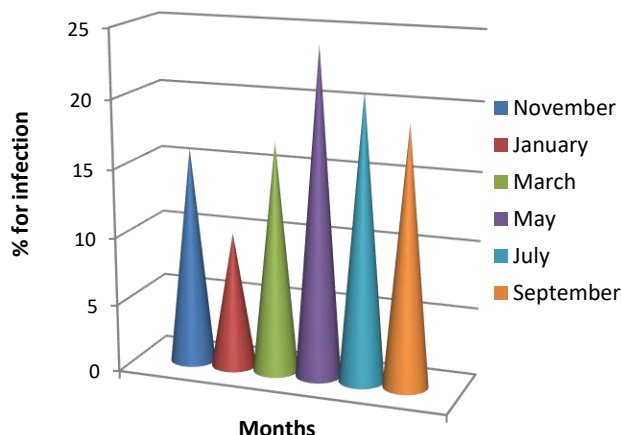


Figure 1. Showcases the percentage of casuarina seedling root rot from November 2020 to September 2021.

Isolation: The results of fungus isolation revealed root rot in *Casuarina equisetifolia* seedlings from various Mosul greenhouses, including those of the Forestry Department, during the surveyed periods. This isolation work highlighted the presence of *F. solani* and *R. solani* fungi, with varying prevalence levels through the isolation process. Figure 2 visually demonstrates the emergence of *F. solani* with the highest isolation rate recorded in July 2021, at 52%. Conversely, the lowest isolation rate for *F. solani* was documented in November 2020, reaching 27%. Similarly, the highest isolation rate for *R. solani* occurred in July 2021, at 35.33%, while the lowest isolation rate was marked in January 2021, at 10.33%.

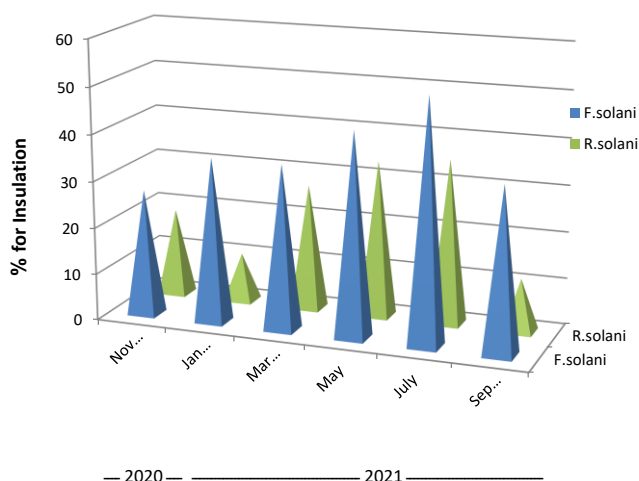


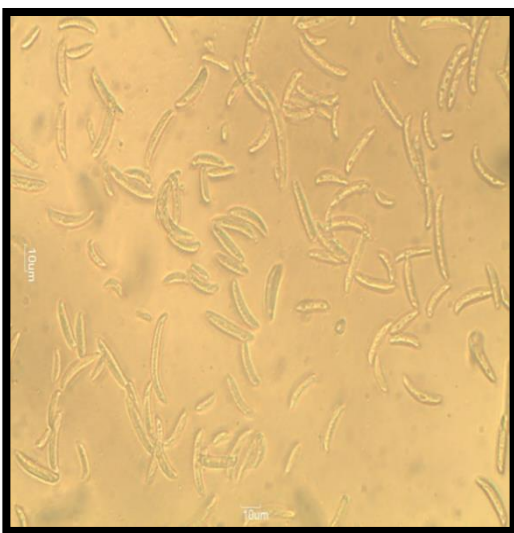
Figure 2. Percentage of root rot fungi isolation in casuarina seedlings.

The analysis underscores the apparent dominance of *F. solani* among casuarina seedlings based on the frequencies of fungal isolation ratios. This dominance can potentially be attributed to the specific thermal and environmental conditions favored by this fungus. *F. solani* is recognized as one of the most prevalent soil fungi, notorious for its pathogenicity across various plant hosts. Its impact manifests as root rot symptoms and the destruction of these plant seedlings, considering its wide distribution in soil (Booth, 1971; Brasileiro *et al.*, 2004). The prevalence of *F. solani* and *R. solani* fungi in casuarina occurrences aligns with findings from the isolation of fungi in different forest nurseries across Iraq. These findings encompass various regions and plant species including *Casuarina*, *Pinus brutia*, *Cupressus Sempervirens horizontalis* and *Thuja Orientalis* (Muhammad, 1987; Al-Obeidi, 2019).

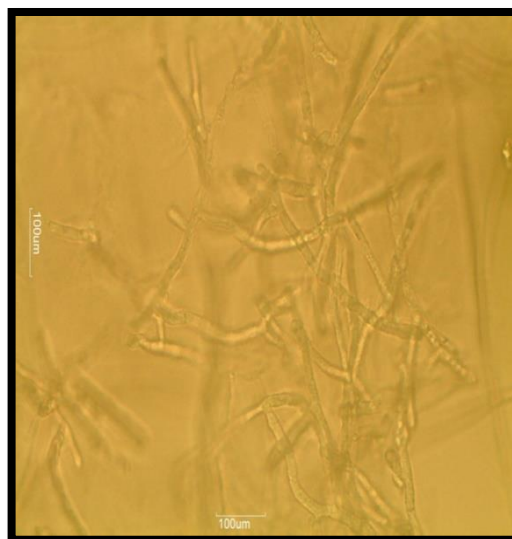
Description and diagnosis of fungus, Mart Fusarium solani: The findings from the diagnostic assessment, as depicted in Figure 3, of the fungus *F. solani* cultivated on PDA nutrient medium—specifically Potato Dextrose Agar—at a temperature of $25^{\circ}\text{C} \pm 2$ over a ten-day period, reveal the emergence of colonies with hues ranging from white to gray. These colonies showcase a vibrant creamy discoloration that in some instances features a subtle purple undertone. Additionally, microscopic scrutiny demonstrates the presence of three types of spores: small conidian spores, also known as Microconidia, which assume an elliptical, cylindrical, or oval shape, measuring $8.2\text{--}15 \times 2.5\text{--}3.6$ micrometers. Larger conidia spores, characterized by their fusiform shape, exhibit dimensions of $35\text{--}37 \times 2.4\text{--}3.4$ μm . The third type is chlamydospores, which may appear as individual entities or in pairs, occasionally accompanied by minor lateral branches. In some cases, these chlamydospores form amidst the central region of fungal filaments. If these characteristics align with the classification keys outlined by various researchers (Booth, 1977; Leslie & Summerell, 2006; Toussoun & Nelson, 1976) the identification process is successful.

Fungus Rhizoctonia solani Khun: Regarding the fungus *R. solani*, as showcased in Figure (4), the diagnostic results highlight the development of colonies on PDA medium. These colonies exhibit a light brown color and mature within eight days at a temperature of $25^{\circ}\text{C} \pm 2$. Notably, their growth speed varies, and the formation of dark-hued sclerotia bodies is also observed. Microscopic examination reveals dense mycelial threads, often confined to a central waist at branching origins. Moreover, barriers are formed in proximity to the evolutionary regions, which aligns with observations made by (Carling *et al.*, 2022).





(A)



(A)



(B)

Figure 3. (A) illustrates the conidia of the *F. solani* fungus under a magnification power of 40X. (B) depicts the shape of the fungus colony.



(B)

Figure 4. displays (A) the mycelium of the *R. solani* fungus at a magnification power of x40. (B) illustrates the shape of the fungus colony.

Table 1. Impact of Artificial Infection by Root Rot Fungi on Infection Percentage and Select Growth Characteristics of Casuarina Seedlings.

Fungi	% infection	Vegetative height (cm)	Stem wet weight (g)	Stem dry weight (g)	Root total length (cm)	Root wet weight (g)	root dry weight (g)
Control treatment without fungi	0	77.33	24.71	11.58	37.5	7.69	5.56
<i>R.solani</i>	C	A	A	A	A	A	A
	25	69.44	11.08	7.32	17	6.3	5.3
<i>F. solani</i>	B	B	BC	B	C	B	B
	41.66	63.44	11.90	6.78	20	6.11	5
	A	C	B	C	B	BC	BC

Similar letter-carrying numbers within a column indicate no significant distinctions between them at a 0.05 probability level.



Evaluating the Pathogenic Impact of Fungi on Casuarina Seedling Growth Characteristics:

Table 1 outlines the outcomes of the disease capability test, revealing that *F. solani* exhibited a higher infection percentage at 41.66%, followed by *R. solani* at 25%. Notable disparities were observed in the influence exerted on all the growth characteristics under examination. *F. solani* significantly affected the average vegetative height of cypress seedlings, yielding a height of 63.44 cm. This was trailed by *R. solani* with a height of 69.44 cm. Concerning the wet weight trait, while there were no significant differences between the two fungi in comparison to the control treatment, *F. solani* showcased a significant impact in diminishing the dry weight of the stem, registering a maximum of 6.78 g. In contrast, *R. solani* demonstrated a lower average effect on the dry weight of the stem, amounting to 7.38 g. The root total length attribute was most influenced by *R. solani*, reaching 17 cm, while *F. solani* exhibited the lowest effect, with a root length of 20 cm. In terms of wet root weight, *R. solani* had an average effect of 6.3 g, which did not significantly differ from the effect of *F. solani*, measuring 6.11 g when compared to the control treatment. Similar trends were noted for the dry root weight trait. In summary, the examined fungi notably impacted various growth characteristics, encompassing vegetative height, root system development, and the dry and wet weight of seedlings, as previously indicated by (Taha *et al.*, 1986; Muhammad, 1987). Pan fungus *F. solani* and *R. solani* have been associated with pronounced effects on the growth attributes of stem height, root length, and dry and wet weights in diverse forest seedlings, including casuarina, as reported in (Agrious, 1997).

The enhanced growth observed in the control treatment is primarily attributed to roots developing within an environment devoid of pathogenic organisms, which would otherwise hinder cell size and undermine water and nutrient absorption efficiency. It is crucial to note that the action of fungi predominantly hinges on enzymes that break down cell walls. Among these enzymes, Polygalacturonase holds

particular significance, targeting the middle lamella and resulting in the softening of root hairs and embryonic stems, in accordance with (Lozovaya *et al.*, 2006). Fungi also exhibit the capacity to release enzymes like Ligninase and peroxidase that degrade host cell wall components, including lignin. The implications of this process extend to the release of fungal toxins and enzymes within these cells, ultimately influencing plant growth traits.

Impact of Alcoholic and Aqueous Leaf Extracts on Fungal Growth Inhibition Rates:

Table 2 presents the outcomes of biological tests conducted on *paulownia tomentosa* leaf extract and its influence on Casuarina seedling root rot fungi. In terms of growth inhibition, it was observed that the fungus *R. solani* was impacted by the second concentration of alcoholic leaf extract, leading to a growth inhibition rate of 57.50%. This rate escalated to 100% at the fourth concentration. Similarly, *F. solani* exhibited a growth inhibition rate of 56.60% for the alcoholic extract at the third concentration and 69.81% at the fourth concentration. The alcoholic leaf extract distinctly affected the growth inhibition rates of both fungi.

Regarding the aqueous extract, the highest inhibition rate for *R. solani* was 35% at the fourth concentration, while the lowest inhibition rate recorded was 2.50% at the first concentration. The limited effectiveness of the aqueous paulownia leaf extract could be attributed to the inadequate dissolution of certain active compounds, as mentioned by (Lawrence *et al.*, 2008). Specific active compounds are capable of interacting with DNA formation and creating ion channels within the vessels of pathogenic fungi. Conversely, the alcoholic leaf extract exhibited significant impact on the growth inhibition rates of both fungi, from the first concentration to the fourth concentration. Although significant differences were evident for all concentrations in comparison to the control treatment, the aqueous extract did not demonstrate significant differences at the first, second, and third concentrations for both fungi. However, its distinction from the control treatment was significant. In

Table 2. Influence of Different Paulownia Leaf Extract Concentrations on Fungus Growth Inhibition Rates.

Extract type	type of Fungus	Percentage of growth inhibition (mm) (%) Concentrations					Average types of extraction	Average type of Fungus
		0%	5%	15%	30%	45%		
Alcohol the leaf extract	<i>F.solani</i>	0	18.86	33.96	56.60	69.81	46.42	30.58
		P	JK	GH	C-F	C-E	A	B
	<i>R. solani</i>	0	36.25	57.50	91.25	100		42.81
		P	GH	C-F	B	A		A
Aqueousthe leaf extract	<i>F.solani</i>	0	9.09	12.72	16.36	27.27	12.29	
		P	MN	KL	JK	IJ	B	
	<i>R. solani</i>	0	2.50	8.75	11.25	35		
		P	M-O	K-M	KL	GH		
average effect of the concentrations		0	16.67	28.23	43.86	58.02		
		P	D	C	B	A		

Similar letters within a column indicate no significant differences at a 0.05 probability level.



terms of averages, the alcoholic extract outperformed the aqueous extract by 46.42%, with the former exhibiting a superiority of 58.02% for the fourth concentration over all others. This could be attributed to the solubility of active compounds in alcohol that possess inhibitory effects on the studied fungi (Otang *et al.*, 2011). The increased concentration of alcoholic extract against pathogenic fungi had effects on fungal cells and filaments, resulting in changes associated with the weakening of the cell wall, responsible for structural integrity (Lawrence *et al.*, 2008). The impact of these extracts increases with escalating concentrations (Paran *et al.*, 1996; Malabidah, 2022). Refer to Figures [5 and 6].



Figure 5. Impact of Alcoholic Paulownia Leaf Extract on Fungal Growth (F.S = F. Solani, R.S = R. Solani).



Figure 6. Impact of Aqueous Paulownia Leaf Extract on Fungal Growth (F.S = F. Solani, R.S = R. Solani).

Conclusions:

1. The presence of root rot disease within Mosul greenhouses during survey months, leading to root decay in various forest tree seedlings, including *Casuarina equisetifolia* L.

2. The concurrent appearance of *F. solani* and *R. solani* fungi accompanying infected *Casuarina equisetifolia* seedling roots, isolated during different months.
3. Prevalence of the pathogenic fungus *F. solani* with the highest isolation ratio.
4. The industrial infection displayed the potential and pathogenicity of casuarina seedling infection by fungi, impacting normal growth traits such as stem length, root length, and dry and wet seedling weights.
5. Variation in the pathogenic potential of fungi, where *F. solani* predominantly influenced natural growth attributes such as plant height and total vegetative dry weight. Meanwhile, *R. solani* singularly affected the length of Casuarina seedling root systems.
6. The alcoholic leaf extract's superiority over the aqueous extract in inhibiting the growth of both *F. solani* and *R. solani* fungi across all employed concentrations.

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Availability of data and material: We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere .

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Consent for publication: All authors submitted consent to publish this research. article in JGIAS.

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